coronate flowers and have been transferred accordingly; but there are still some species (e.g., *Astephanus geminiflorus* Decne., *A. multiflorus* T. Mey.) that have not been transferred yet because generic limits in New World Asclepiadeae are still very poorly understood.

Liede (1994) noticed that *Astephanus* s. str. and the closely related *Microloma*, both of which lack a corona, have colorless latex and long, non-verrucose hairs on the adaxial corolla surface, and that they shared this combination of the two features with nine other, coronate genera: *Blyttia* Arn., *Diplostigma* K. Schum., *Goydera* Liede, *Oncinema* Arn., *Pentatropis* R. Br., *Pleurostelma* Baill., *Rhyncharrhena* F. Muell., *Schistostephanus* Hochst. ex Benth., and *Tylophoropsis* N. E. Br. Since these two features are not found in combination elsewhere in Asclepiadeae, she concluded that they most likely represent an apomorphy for this group, and therefore added these nine genera to the Astephaninae (Liede, 1994).

In her synopsis of Asclepiadeae as a whole, Liede (1997) added five more genera to the Astephaninae (*Emicocarpus* K. Schum., *Eustegia* R. Br., *Seshagiria* Ansari & Hemadri, *Tylophora*, and *Vincetoxicum* Medik.), based on the presence of one or both of the following features: long, slender hairs on the adaxial surface of the corolla and sparse clear latex. Traditionally, *Tylophora* has not even been considered to be a member of Asclepiadeae because Schumann (1895) misinterpreted the position of the pollinia in the anther sacs. Schumann's (1895) Tylophoreae K. Schum., except for *Tylophora*, the nomenclatural type, comprise exclusively taxa today considered to be members of Marsdenieae and Stapelieae. Swarupanandan et al. (1996) discussed the position of pollinia in detail, coming to the conclusion that *Tylophora* is a member of Asclepiadeae. Additionally he found (Swarupanandan, 1996) that *Tylophora* also possesses the elongated style typical for Asclepiadeae. Recent molecular studies (Sennblad, 1997; Civeyrel et al., 1998) confirmed its position in Asclepiadeae. Following these results, Liede (1997) put *Tylophoropsis* N. E. Br. into synonymy under *Tylophora*, since it only differs by its pendent pollinia (in contrast to the horizontal ones in *Tylophora*), which does not constitute a fundamental difference.

Only two of the nine genera comprising Schumann's (1895) Astephaninae (*Astephanus* and *Microloma*) are currently recognized in this subtribe. Of the remaining seven genera, *Henrya* has been put into synonymy under *Tylophora*, and *Esmeraldia* into synonymy under *Metastelma*, and the remainder have been transferred as good genera to other Asclepiadeae subtribes by Liede (1997). *Amblystigma* and *Mitostigma* were transferred to the Oxypetalinae since both possess the combination of a corolla tube at least half as long as the total corolla length and a very conspicuous long stylar head, which are apomorphies of Oxypetalinae. The monotypic *Nautonia* agrees in habit, floral structure, and fruit and seed morphology with both *Metastelma*, type genus of Metastelminae, and the closely related *Ditassa*, which differs from *Metastelma* only in that most of its species have a double instead of a simple staminal corona. Even though some species of *Hemipogon* look different in habit and corolla shape, the genus is linked to the Metastelminae by species such as *H. luteus* E. Fourn., and all species agree with Metastelminae very well in fruit and seed morphology (Liede et al., unpublished data). Lastly, the monotypic Asian *Adelostemma*, which was originally described under *Cynanchum* L., and was only excluded from *Cynanchum* because its lack of a corona, agrees with *Cynanchum* s. str. in all other features. Thus *Nautonia*, *Hemipogon*, and *Adelostemma* were all transferred to the Metastelminae.

Liede (1994) conducted a cladistic analysis of the Astephaninae (without the five genera added in Liede, 1997) based on 22 morphological characters. However, this analysis was flawed because *Tylophora*, then still considered a Marsdenieae, was used as the outgroup; while later research showed that it actually belongs to the Asclepiadeae (Swarupanandan et al., 1996) and is congeneric with *Tylophoropsis*, which had been recognized as member of the Astephaninae by Liede (1994), so that *Tylophora* should have been considered a member of the ingroup (Liede, 1997).

Liede's (1994, 1997) concept of Astephaninae was criticized by Bruyns (1999a), who examined a living plant of *Seshagiria* and found it to contain white latex, whereas Liede (1997) had no information on this character. Bruyns (1999b) also conducted a cladistic study on almost the same range of genera, but excluded *Seshagiria*, though he did not exclude it formally from the subtribe, and found *Eustegia* and *Emicocarpus* so closely related that he treated them as one unit in his cladistic analysis. He (Bruyns, 1999b) suggested numerous embellishments to the matrix of morphological characters given in Liede (1994). However, these characters are not necessarily any more useful for cladistic analysis. For example, character 9 (coralline corona present or absent) in Bruyns (1999b) is irrelevant because a coralline corona is absent throughout the ingroup. Bruyns's (1999b) choice of the distantly related genus *Secamone* R. Br. as an outgroup might contribute to the isolated basal position of *Tylophora* in his strict consensus tree (Bruyns, 1999b). In contrast, Liede (1996) found support for a close relationship between *Tylophora* and *Vincetoxicum* in the occurrence of alkaloids and of 14, 15-seco-pregnanes in both genera, but nowhere else in Asclepiadeae, as far as known. This close relationship has been confirmed both by *mat*K sequence data (Civeyrel et al., 1998) and *rbc*L sequence data (Sennblad, 1997).

The present paper investigates the circumscription of the Astephaninae sensu Liede (1994, 1997) by a molecular marker, the *trn*T-L spacer, *trn*L intron, and *trn*L-F spacer.

Materials and Methods

Taxa

Material was available of all genera of Astephaninae sensu Liede (1994, 1997) except three (*Emicocarpus, Rhyncharrhena, Seshagiria;* Table 1). *Gymnema* R. Br. and *Cionura* Griseb., members of the Marsdenieae, and *Ceropegia* L. and *Stapelia* L., members of the Ceropegieae, were chosen as outgroups belonging to different tribes. In the Asclepiadeae, a wide range of different genera was included, focusing on genera and species without a corona. They are listed in Table 1 (refer here for authors of species) according to their classification by Liede (1997).

DNA extraction and PCR

DNA was isolated from fresh or dried leaf tissue according to Doyle and Doyle (1987). PCR primers and protocol for the plastid *trn*T-*trn*L and *trn*L-*trn*F spacers as well as the *trn*L intron follow Taberlet et al. (1991). Sequences were obtained on an ABI Prism Model 310 Version 3.0 sequencer. Of the 43 taxa, 36 have been sequenced for this study; the remaining seven sequences had been deposited at EMBL in the course of earlier studies of the author (for accession numbers, see Table 1).

Data analysis

Sequences were pre-aligned with Perkin Elmer Sequence Navigator Version 1.0.1; the alignment was cleaned manually. The sequence alignment (available from the author) comprises 43 taxa and 2088 characters (1076 sequence characters and 18 indels in the *trn*T-*trn*L intron (primers a and b), 547 sequence characters and 9 indels between the two *trn*L-exons (primers c and d), and 432 sequence characters and 6 indels in the *trn*L-*trn*F intron (primers e and f)); 45 data cells are unknown and were coded as missing characters.

Phylogenetic analysis and tests for clade support were performed using PAUP version 4.0d65 (PPC; Swofford, 1998), on a Macintosh Powerbook G3. Indels were coded as "missing" characters throughout; possibly parsimony-informative indels were coded separately following the "simple gap coding" method of Simmons and Ochoterena (2000). In two areas with very irregular and potentially ambiguous indel pattern in the *trn*L-F spacer (bp 340–408, 693–758), no separate indel coding was performed. Different lengths of poly-chains of more than 5 bp have not been coded as indels either because the length of these chains has been found to be variable even within the same species (Liede, unpublished data).

For parsimony analysis, first all sequence characters were analyzed. Then the 33 separately coded indels were added. Heuristic search for both data sets was conducted in two steps: first, starting trees were obtained setting addition sequence at "random" and 1000 replicates, "MulTrees" and "Steepest descent" off. Then, these starting trees were subjected to TBR branch swapping, "MulTrees" on, "Steepest descent" off.

Bootstrap search (1000 replicates) was conducted under the "fast" stepwise addition type of search. Jackknife resampling (1000 replicates) was set to 50% deletion, and "Jac" resampling; the other settings were identical to the bootstrap settings.

Results

Parsimony analysis of all sequence characters (yielding 202 parsimony-informative characters) results in 104 most parsimonious trees (l = 611, CI = 0.8494, RI = 0.8777, RC = 0.7455). Adding the indels yields 234 parsimony informative characters (indel 8 of the *trn*T-L spacer is not parsimony informative), and analysis results in 40 most parsimonious trees (l = 670, CI = 0.8239, RI = 0.8671, RC = 0.7144). The strict consensus tree resulting from both analyses is shown in Figure 1.

In both analyses, the ingroup splits into two major, well-supported clades, the *Astephanus*-clade and the *Tylophora*-clade, though the position of taxa within the *Tylophora*-clade is less well established. The topology of the strict consensus resulting from the addition of the indels changes only the position of the *Pentatropis*-clade from the base of the *Tylophora*-clade to an unresolved subclade of the *Tylophora*-clade (dashed line in Fig. 1) and distinguishes two unsupported subclades of the

Table 1. Voucher and locality information for plant material used in this study. All taxa belong to the Apocynaceae–Asclepiadoideae. * indicates species without a corona.

Species	Origin	Voucher	EMBL Accession No. *trn*T-L spacer *trn*L intron *trn*L-F spacer
OUTGROUPS			
Marsdenieae			
Cionura erecta (R. Br.) Griseb.	Turkey	*Heyne 120* (UBT)	AJ410172 AJ410173 AJ410174
Gymnema sylvestre (Retz.) Schult.	Cameroon	*Meve 919* (UBT)	AJ402118 AJ402137 AJ402142
Ceropegieae			
Ceropegia nilotica Kotschy	Kenya	*Masinde 836* (MSUN)	AJ402117 AJ402138 AJ402141
Stapelia glanduliflora Masson	South Africa	*Albers & Meve 04* (MSUN)	AJ402127 AJ402128 AJ402151
Asclepiadeae–Asclepiadinae			
Asclepias syriaca L.		ex hort. Münster; in cult. Münster	AJ410178 AJ410179 AJ410180
Gomphocarpus physocarpus E. Mey.	South Africa	*Nicholas 2829* (UDW)	AJ290877 AJ290876 AJ290875
Pergularia daemia (Forssk.) Chiov.	Tanzania	*Masinde 888* (UBT)	AJ290891 AJ290892 AJ290893
Asclepiadeae–Metastelminae (Old World)			
Biondia henryi (Warb. ex Schltr. & Diels) Tsiang & P. T. Li	China	*Deng 90203* (MO)	AJ410190 AJ410191 AJ410192
Cynanchum auriculatum Royle ex Wight	China	ex hort. Nanking (UBT)	AJ410196 AJ410197

Table 1. Continued.

Species	Origin	Voucher	EMBL Accession No. *trn*T-L spacer *trn*L intron *trn*L-F spacer
Cynanchum ellipticum (Harv.) R. A. Dyer	South Africa	*Liede 2933* (UBT)	AJ410198 AJ290847 AJ290846
Pentarrhinum insipidum E. Mey.	South Africa	*Liede 2940* (UBT)	AJ290845 AJ410232 AJ410233 AJ410234
Asclepiadeae–Metastelminae (New World)			
*"*Astephanus*" *geminiflorus* Decne.	Chile	*Heyne 103* (MSUN)	AJ410181 AJ410182 AJ410183
*Ditassa grazielae (Fontella & Marquete) Rapini ined.	Brazil	*Omlor 147* (MJG)	AJ410202 AJ410203 AJ410204
**Grisebachiella hieronymi* Lorentz	Argentina	*Liede & Conrad 3052* (MSUN, ULM)	AJ410211 AJ410212 AJ410213
Metastelma schaffneri A. Gray	Mexico	*Liede & Conrad 2962* (UBT)	AJ410214 AJ410215 AJ410216
**Nautonia nummularia* Decne.	Argentina	*Liede & Conrad 3031* (ULM)	AJ410226 AJ410227 AJ410228
Asclepiadeae–Oxypetalinae			
**Melinia candolleana* (Hook. & Arn.) Decne.	Argentina	*Liede & Conrad 3055* (ULM)	AJ410175 AJ410176 AJ410177
**Melinia parviflora* (Malme) A. Krapovickas & S. Cáceres Moral	Argentina	*Liede & Conrad 3113* (UBT)	AJ410223 AJ410224 AJ410225
Schistogyne sylvestris Hook. & Arn.	Argentina	*Liede & Conrad 3024* (K, MO, MSUN, ULM)	AJ410244 AJ410245 AJ410246

Table 1. Continued.

Species	Origin	Voucher	EMBL Accession No. *trn*T-L spacer *trn*L intron *trn*L-F spacer
INGROUP (Asclepiadeae–Astephaninae sensu Liede, 1994, 1997)			
**Astephanus neglectus* Schltr.	South Africa	*Goldblatt 2042* (MO)	AJ410184 AJ410185 AJ410186
**Astephanus triflorus* R. Br.	South Africa	*Williams 659 (MO)*	AJ410187 AJ410188 AJ410189
Blyttia fruticulosum (Decne.) D. V. Field	Kenya	*Liede & Newton 2946* (UBT)	AJ410193 AJ410194 AJ410195
Diplostigma canescens K. Schum.	Kenya	*Liede & Newton 3214* (UBT)	AJ410199 AJ410200 AJ410201
Eustegia minuta (L.f.) N. E. Br.	South Africa	*Bruyns 4357* (K; MWC 3291)	AJ410205 AJ410206 AJ410207
Goydera somaliense Liede	Somalia	*Thulin & Bashir 6882* (UPS)	AJ410208 AJ410209 AJ410210
**Microloma sagittatum* R. Br.	South Africa	*Meve & Liede 616* (MSUN)	AJ410217 AJ410218 AJ410219
**Microloma tenuifolium* K. Schum.	South Africa	*Albers s.n.* (MSUN)	AJ410220 AJ410221 AJ410222
Oncinema lineare (L.f.) Bullock	South Africa	*Bruyns s.n.* (K; MWC 3290)	AJ410229 AJ410230 AJ410231
Pentatropis madagascariensis Decne.	Madagascar	*Liede 2749* (UBT)	AJ410235 AJ410236 AJ410237
Pentatropis nivalis (J. F. Gmel.) D. V. Field & J. R. I .Wood	Kenya	*Meve 949* (UBT)	AJ410238 AJ410239

Table 1. Continued.

Species	Origin	Voucher	EMBL Accession No. *trn*T-L spacer *trn*L intron *trn*L-F spacer
Pleurostelma cernuum (Decne.) Bullock	Tanzania	*Liede & Meve 3377* (UBT)	AJ410240 AJ410241 AJ410242
Schizostephanus alatus Hochst. ex K. Schum.	Kenya	*Noltee s.n. sub IPPS 8111* (UBT)	AJ410243 AJ410247 AJ410248
Tylophora anomala N. E. Br.	Cameroon	*Meve 916* (K, UBT)	AJ410249 AJ410250 AJ410251
Tylophora apiculata K. Schum.	Kenya	*Robertson 7016* (UBT)	AJ410252 AJ410253 AJ410254
Tylophora flanaganii Schltr.	South Africa	*Nicholas 2839* (UDW)	AJ410255 AJ410256 AJ410257
Tylophora flexuosa R. Br. var. perrottetiana (Decne.) Schneidt ined.	Philippines	*Liede 3252* (UBT)	AJ410258 AJ290915 AJ290916
Tylophora heterophylla A. Rich.	Kenya	*Liede & Newton 3155* (UBT)	AJ290917 AJ410259 AJ410260
Tylophora indica (Burm. f.) Merrill	India	*Bruyns s.n.* (UBT)	AJ410261 AJ410262 AJ410263
Tylophora sylvatica Decne.	Africa (ex hort.)	*Valck s.n.* (UBT)	AJ410264 AJ410265 AJ410266
Vincetoxicum atratum Morr. & Decne.	China	*Schneidt 96-137* (ABD)	AJ410267 AJ410268 AJ410269
Vincetoxicum carnosum Benth.	Borneo	*Schneidt 95-97* (ABD, L)	AJ410270 AJ410271 AJ410272 AJ410273

Table 1. Continued.

Species	Origin	Voucher	EMBL Accession No. *trn*T-L spacer	*trn*L intron	*trn*L-F spacer
Vincetoxicum hirundinaria Medic.	Germany	*Meve s.n.* (UBT)	AJ410274	AJ410275	AJ410276
Vincetoxicum stocksii S. I. Ali & S. Khatoon	Pakistan	*Ali & Khatoon s.n.* (GA)	AJ410277	AJ410278	AJ410279

Tylophora-clade (dotted lines in Fig. 1). In both analyses, *Eustegia* forms the most basal clade in Asclepiadinae, followed by the *Astephanus*-clade. The New World Metastelminae (including the three representatives of Oxypetalinae as a subclade) follow and are equally well supported. The main clades that follow are the Old World Metastelminae (including *Schizostephanus*), the Asclepiadinae, and, last, the *Tylophora*-clade, which comes out in the most derived position.

Discussion

The genera placed in Astephaninae by Liede (1994, 1997) are not monophyletic according to the results of the cpDNA analyis.

Schizostephanus is more closely related to *Pentarrhinum* and the Old World species of *Cynanchum* than to other Astephaninae genera. *Schizostephanus* shares with *Cynanchum* the highly fused corona of staminal and interstaminal parts as well as the reniform leaf bases and possesses thus the characters listed as synapomorphies for the Metastelminae Endl. ex Meisn. sensu Liede (1997), to which it is consequently transferred.

Eustegia shows no close affinity, either morphologically or molecularly, to any other genus included in the analysis and takes a basal position within the tribe Asclepiadeae. Bruyns (1999b) has examined the close relationship of *Eustegia* and *Emicocarpus*, in particular with reference to their unique 3-seriate corona. The isolated position of *Eustegia* and *Emicocarpus* argued for by Bruyns (1999b) is supported by the present results at least for *Eustegia*. Both genera are monotypic, and both occupy a very restricted distribution area, *Eustegia* in the Western Cape, and *Emicocarpus* around Maputo (Mozambique), suggesting that these two genera might be relics of a once more widespread and diverse group of Asclepiadeae.

The three southern African genera *Astephanus*, *Microloma*, and *Oncinema* form a clade with 100% bootstrap and jackknife support in the present analysis. Bruyns and Linder (1991) listed "similarly shaped, small subcoriaceous leaves and slender climbing habit and the similarly elongated style apex" as well as clear latex as common characters of these three genera. While none of these characters alone is unique in Asclepiadeae, the combination of all three can be used to characterize the Astephaninae-clade. All species of *Microloma* investigated possess a chromosome number of $x = 10$ (Albers et al., 1993), while the vast majority of Asclepiadoideae, 96 of the 104 genera studied, possess $x = 11$ (Albers & Meve, 2001 this volume).

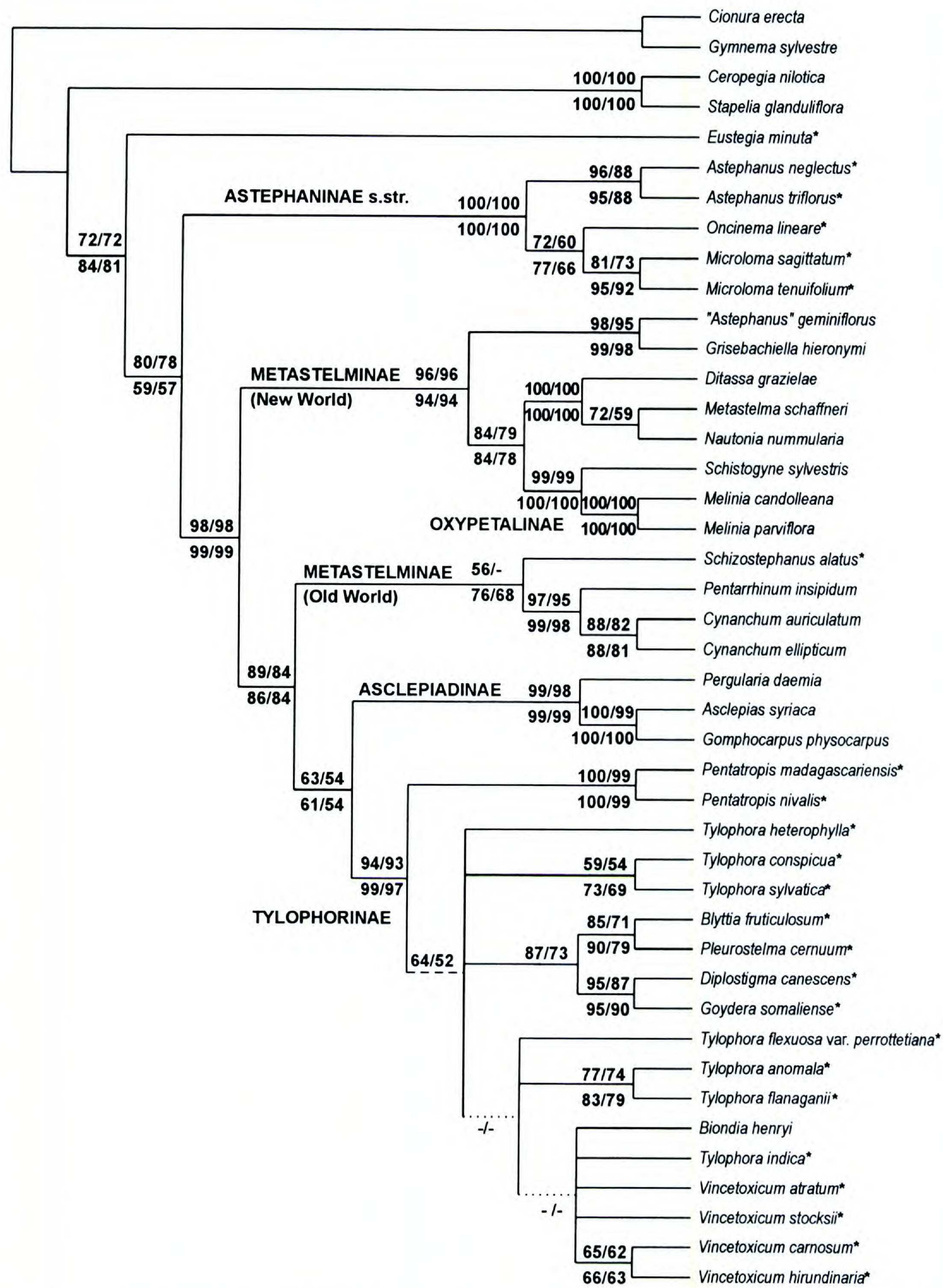

Figure 1. Strict consensus tree of the 104 most parsimonious trees (l = 611, CI = 0.8494, RI = 0.8777, RC = 0.7455) resulting from parsimony analysis of all sequence characters and of the 40 most parsimonious trees (l = 670, CI = 0.8239, RI = 0.8671, RC = 0.7144) resulting from analysis of all sequence characters and all indels. Asterisks denote taxa included in Astephaninae sensu Liede (1997). Dotted lines indicate clades not retrieved in the analysis without the indels; the dashed line indicates a clade not retrieved in the analysis including the indels. Numbers indicate bootstrap/jackknife values and refer to the analysis without the indels above branches and with the indels below the branches.

Unfortunately, there are no cytological data available on this potential synapomorphy for *Astephanus* and *Oncinema*. With *Astephanus*, this clade includes the nomenclatural type of the subtribe Astephaninae. The Astephaninae s. str. therefore include only three genera: *Astephanus*, *Microloma*, and *Oncinema*. This subtribe is restricted to the Old World, only occurring in southern Africa; the results of the present analysis confirm that none of the corona-less New World taxa is a member of *Astephanus* R. Br. Records of *Microloma incanum* Decne. in Madagascar (Meve & Liede, 1995) might point to a once more widespread distribution of Astephaninae s. str. in Africa, but the material is too scanty to postulate such an extended distribution area with certainty. The Astephaninae s. str. occupy the second most basal position in the Asclepiadeae (Fig. 1). As both most basal clades (*Eustegia* and Astephaninae s. str.) occur in southern Africa, it can be speculated that the origin of Asclepiadeae probably lies in the southern African area.

New World Metastelminae (sensu Liede, 1997) (including Oxypetalinae), the Asclepiadinae, and the Old World Metastelminae form monophyletic clades between Astephaninae s. str. and a clade comprising the remainder of the genera attributed to Astephaninae sensu Liede (1994, 1997) together with *Biondia*, which was formerly considered a member of Metastelminae. The split of Metastelminae sensu Liede (1997) into an Old World and a New World clade has been observed in an analysis of the genera *Sarcostemma* s.l. (Liede & Täuber, 2000) and *Cynanchum* (Liede & Täuber, in prep.), and is discussed in depth in the latter paper. The close relationships between Oxypetalinae and the New World clade of Metastelminae is at present under study (Liede & Goyder, unpublished results).

Biondia, *Blyttia*, *Diplostigma*, *Goydera*, *Pentatropis*, *Pleurostelma*, *Tylophora*, and *Vincetoxicum* form a well-supported clade in the analysis of sequence characters alone, and their close relationship is supported by the indel pattern, as the inclusion of indels raises both bootstrap and jackknife values (Fig. 1). Resolution within this clade is low, with the exception of the well-supported subclade formed by the four small African genera *Blyttia*, *Diplostigma*, *Goydera*, and *Pleurostelma*. The basal position of *Pentatropis* is weakly supported in the analysis of sequence characters alone, and the *Pentatropis*-clade forms an unresolved subclade of the main clade in the analysis including the indels (Fig. 1). As a corollary, *Vincetoxicum carnosum* Benth. always appears in the unresolved *Tylophora*-clade and should not be transferred to *Pentatropis* as was suggested earlier due to its morphology (Liede, 1994). Common morphological characters of the genera in the *Tylophora*-clade include rather small, inconspicuous flowers with a gynostegial corona of five separate staminal parts, and small, often disk-shaped pollinia attached to the corpusculum via cylindrical caudicles. Latex is clear in almost all taxa except for some species of *Tylophora*, which have white or yellowish latex. Non-verrucose hairs on the adaxial corolla surface are also present (Liede, 1994, 1997), mainly at the entrance of the tube. *Rhyncharrhena*, one of the genera for which no sequenceable material could be obtained, also shows these characters. Its only species was originally described under *Pentatropis* (*P. linearis* Decne.), and while the characters listed by Wilson (1980) may warrant its recognition as a distinct genus, its morphology indicates a position between *Pentatropis* and *Tylophora*, in particular with regard to corona and inflorescence structure. Therefore, *Rhyncharrhena* is tentatively placed in the Tylophorinae.

The *Tylophora*-clade has been analyzed for a second marker, ITS (Liede et al., in press), for which the same pattern has been found with a strongly supported clade and weak internal resolution. An attempt to align ITS sequences of the three Astephaninae s. str. genera with those of *Tylophora* and its allies failed (Liede, unpublished data), which is not surprising considering that ITS in general has a much faster rate of change than the cpDNA regions analyzed here. For the members of the *Tylophora*-clade, the name Tylophorinae K. Schum. is appropriate, even though Schumann (1895) used it to circumscribe a set of genera now classified as Marsdenieae and Ceropegieae except for the type genus, *Tylophora* (Liede & Albers, 1994). While it is unfortunate that *Tylophora* is the only genus common to Tylophoreae sensu Schumann (1895) and Tylophorinae as circumscribed here, Article 47 and Recommendation 19A.2 of the ICBN (Greuter et al., 2000) indicate that this is the correct name for the taxon.

The Tylophorinae, with the two species-rich genera *Tylophora* and *Vincetoxicum*, are distributed throughout the Old World, with a center of generic diversity in East Africa. Contrary to traditional views that taxa with a very simple floral structure are primitive in Asclepiadeae, Tylophorinae occupy an advanced position within the Asclepiadeae, which comes out as the crown clade in our study. Sennblad (1997), Civeyrel et al. (1998), Fishbein (2001 this volume), and Potgieter and Albert (2001 this volume) have analyzed a smaller number of Asclepiadeae taxa. In the *rbc*L study of Sennblad, the *Tylophora*/*Vincetoxicum*-clade comes out as sister to an Oxypetalinae/Gonolobinae-clade in the most derived position. The strange position of "*Cynanchum*" in this study (Sennblad, 1997) is explained by the choice of a New World representative (*C. serpyllifolium* Kunth) that is not a member of *Cynanchum* s. str. (Liede & Täuber, in prep.). *Schizostephanus* (a member of Old World Metastelminae) and *Asclepias*/*Calotropis* (Asclepiadinae) are unresolved sisters to the Tylophorinae/Oxypetalinae/Gonolobinae-clade (Sennblad, 1997), for which, unfortunately, no support values are given. In the *mat*K study of Civeyrel et al. (1998) the *Tylophora*/*Vincetoxicum*-clade again comes out as sister to an Oxypetalinae/Gonolobinae-clade in a more derived position than *Pergularia* (Asclepiadinae) and *Pentarrhinum* (Old World Metastelminae). In the *mat*K study of Fishbein (2001) the *Tylophora*/*Vincetoxicum*-clade forms one of the unresolved Asclepiadeae-clades. In the combined *trn*L-F spacer, *trn*L intron and morphological fruit character study of Potgieter and Albert (2001), the three unresolved clades *Oxystelma* (one species), Tylophorinae (four species), and Asclepiadinae (seven species) take the most derived position in Asclepiadeae. From these studies it becomes clear that Tylophorinae are one of the most derived groups of Asclepiadeae, so that their rather simple floral structure has to be regarded as an advanced rather than a primitive character.

The fact that Tylophorinae most likely represent an advanced group of genera is underlined by the distribution of *Vincetoxicum*. Of all Asclepiadoideae (and even Apocynaceae), *Vincetoxicum* has radiated furthest to the north (as far as Sweden), away from the sub-tropical African center of origin of the subfamily (Good, 1951). *Vincetoxicum* possesses a remarkable potential to expand range distribution, probably due to its capacity of self-fertilization, unusual in Asclepiadoideae (Lumer & Yost, 1995), and its rapid spread throughout the United States and Canada after its accidental introduction in several places along the east coast of North America in the second half of the last century has been well documented (e.g., Sheeley & Raynal, 1996).

No material for sequencing was available for *Seshagiria*, a recently described Indian genus of doubtful affinity (Ansari & Hemadri, 1971a, 1971b). Its floral structure is strongly reminiscent of that in some members of *Pentatropis* (e.g., *P. oblongifolia* (Cost.) Liede), but its stout, verrucose fruits are otherwise unknown in Tylophorinae. Bruyns (1999a) has pointed out that *Seshagiria* has white latex, but neither he (Bruyns, 1999a) nor Ansari and Hemadri (1971a, 1971b) have any suggestions as to the relationships of this rare monotypic genus. Thus, for the time being, *Seshagiria* is considered as a genus *incertae sedis* in the Asclepiadeae.

Appendix 1 presents a corollary classification of the Asclepiadeae.

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APPENDIX 1. Corollary taxonomic changes within the Asclepiadeae.

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Metastelma R. Br., Asclepiadeae 41. 1810. TYPE: *Metastelma parviflorum* (Sw.) R. Br. ex Schult.

Schizostephanus Hochst. ex K. Schum., Bot. Jahrb. Syst. 17: 139. 1893. TYPE: *Schizostephanus alatus* Hochst. ex K. Schum.

ASTEPHANINAE Endl. ex Meisn., Pl. Vasc. Gen. 1: 257, 266; 2: 174. 1840. TYPE: *Astephanus* R. Br.

Astephanus R. Br., Asclepiadeae 43. 1810. TYPE: *Astephanus triflorus* (L. f.) R. Br. ex Schult., in Roem. & Schult., Syst. Veg. 6: 122. 1820.

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ELUCIDATING DEEP-LEVEL PHYLOGENETIC RELATIONSHIPS IN SAXIFRAGACEAE USING SEQUENCES FOR SIX CHLOROPLASTIC AND NUCLEAR DNA REGIONS[1]

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Abstract

To elucidate relationships at deep levels within Saxifragaceae we analyzed phylogenetically a data set of sequences for six DNA regions, four representing the chloroplast genome (*rbc*L, *mat*K, *trn*L-*trn*F, *psb*A-*trn*H) and two from the nuclear genome (ITS and expansion segments of the 26S rDNA). A total of 6676 bp was aligned per taxon, 4559 bp and 1878 bp from the chloroplast and nuclear genomes, respectively. Chloroplast and nuclear trees agreed closely, prompting analysis of a combined, six-gene data set. Application of both parsimony and maximum likelihood methods yielded similar topologies. The use of different ITS alignments and the exclusion of hard-to-align ITS regions had little impact on either the final nuclear-based topology, or the shortest trees from the analysis of six genes. The affinities of two monotypic genera (*Saxifragella* and *Saxifragodes*) endemic to Tierra del Fuego were elucidated. *Saxifragella* is an early branching member of the North Temperate genus *Saxifraga* s. str.; *Saxifragodes* is sister to *Cascadia*, a genus endemic to Oregon and Washington. Long-distance dispersal from east Asia or western North America to South America may have played an important role in forming these and other similar disjunctions in the family. A number of well-supported clades are present, including *Saxifraga* s. str., *Micranthes*, *Saxifragopsis/Astilbe*, *Chrysosplenium/Peltoboykinia*, and the *Boykinia* and *Heuchera* groups. The use of additional characters has provided greatly increased resolution and internal support at deep levels. Saxifragaceae comprise two major lineages: *Saxifraga* s. str. (including *Saxifragella*) and all other genera of the family (the heucheroids). This major split is accompanied by general biogeographical and morphological differences. Whereas *Saxifraga* s. str. is largely arctic to alpine in occurrence, the heucheroid clade is largely temperate in distribution. *Saxifraga* s. str. has a relatively uniform floral morphology (generally actinomorphic; 5 sepals, 5 petals, 10 stamens, 2 carpels), whereas the heucheroid clade encompasses actinomorphic and zygomorphic forms, as well as variation in the number of sepals, petals, stamens, and carpels. Deep-level relationships within both *Saxifraga* s. str. and the heucheroid clade are well resolved and supported. A phylogenetic classification of the family is provided.

Key words: molecular systematics, phylogeny, Saxifragaceae, taxonomy.

Saxifragaceae are a eudicot family of approximately 30 genera of herbaceous perennials, about half of which are monotypic (Table 1). The largest genera include *Heuchera* (about 50 species; Rosendahl et al., 1936), *Chrysosplenium* (57 species; Hara, 1957), a narrowly defined *Saxifraga* (over 300 species; Gornall, 1987; Webb & Gornall, 1989), and the *Micranthes* clade (= *Saxifraga* sect. *Micranthes*; approximately 70 species; Gornall, 1987; Webb & Gornall, 1989). Although a modest sized family, members of Saxifragaceae have served as important models for studies of autopolyploid speciation (reviewed in Soltis & Soltis, 1999; Segraves & Thompson, 1999), coevolution, and geographic mosaic speciation (e.g., Thompson, 1994; Thompson & Pellmyr, 1992). Members of Saxifra-

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